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(54) Title: USE OF A DEXTRIN GLYCOSYL TRANSFERASE IN BAKING

(57) Abstract

A dough- or bread-improving composition comprising an effective amount of a DGTase, optionally in admixture with other enzymes, as well as the use of the composition in the preparation of dough and baked products.

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USE OF A DEXTRIN GLYCOSYL TRANSFERASE IN BAKING

FIELD OF THE INVENTION

The present invention relates to a bread-improving or dough-improving composition comprising a dextrin glycosyl transferase (DGTase), as well as to a method of preparing a dough and/or a baked product by use of the composition and/or the enzyme.

BACKGROUND OF THE INVENTION

In the bread-making process it is known to add bread-improving and/or dough-improving additives to the bread dough, the action of which, inter alia, results in improved texture, volume, flavour and freshness of the bread as well as improved machinability of the dough.

In recent years a number of enzymes have been used as dough and/or bread improving agents, in particular enzymes which act on components present in large amounts in the dough. Examples of such enzymes are found within the groups of amylases, proteases and cellulases.

Pentosanases, such as xylanases, have gained considerable importance for use in the preparation of bread and baked products; in particular, to increase the volume and improve the anti-staling potential of bread and other baked products.

For instance, EP 396 162, EP 493 850 and EP 487 122 relate to bread improvers, deep-frozen dough and a fat-free pastry mix, respectively, comprising xylanase optionally in combination with other enzymes. WO 91/18977 discloses a method of preparing a pentosanase-containing preparation having increased baking activity.

EP 687 414 discloses the use of a cyclodextrin glucano-transferase to obtain increased loaf volume and improved flavour.

Much attention has also focused on developing methods for the preparation of bread which is capable of staying fresh for a longer period of time and thus exhibits an increased

resistance to staling. Various starch-modifying enzymes have been suggested for use as anti-staling agents. In JP 62-79745 and JP 62-79746 thermostable β-amylases are described as useful in providing an antistaling effect to baked products, EP 412 607 describes the use of a thermostable α-1,4-exoglucanase or α-1,6-endoglucanase (e.g., pullulanase, amyloglucosidases or β-amylases) as an antistaling agent, and EP 494 233 describes the use of a thermostable maltogenic α-amylase as an antistaling agent.

DGTase (4-α-glucanotransferase or dextrin glycosyltransferase; EC.2.4.1.25) is an enzyme which is believed to catalyse the transglycosylation reaction of maltooligosaccharides. The enzyme catalyses the transfer of a glucosyl or maltooligosyl unit from the non-reducing end of a donor molecule to the non-reducing end of an acceptor molecule, such as glucose or a 1,4-α-D-glucan, resulting in the formation of compounds containing cyclic structures.

In EP 675 137 the use of a D-enzyme (i.e., a DGTase) is described for the production of glucans from a linear α-1,4-glucan or a saccharide containing it, which glucans comprise at least one cyclic structure that has a DP of 14 or more. The resulting glucans are stated as being highly soluble in water, free from retrogradation and useful for a wide variety of purposes and products, including as constituents for food or beverage products, for infusion solutions, for plastics, or for use in inclusion or adsorption of various materials. The glucans are further stated to be useful as anti-retrogradation agents for starch, however no specific use of such anti-retrogradation agents is mentioned.

Branching enzyme (or 1,4-α-glucan branching enzyme, EC.2.4.1.18) is a transferase which is involved in the formation of α-1,6-branches of starch and similar glucans. The enzyme has been isolated from a number of plant and microbial sources and several cloned branching enzymes have been described. More specifically, Zevenhuizen (1964, Biochim. Biophys. Acta 81, 608-611) discloses a branching

enzyme isolated from *Arthrobacter globiformis;* US 4,454,161, a branching enzyme from *Bacillus megaterium;* Walker and Builder (1977, Eur. J. Biochem. 20, 246-253), a branching enzyme from *Streptococcus mitis;* Steiner and Preiss (1977, J. Bacteriol. 129, 246-253), a branching enzyme from *Salmonella typhimurium;* Fredrick, J. (1978, Thermal Biol. 3, 1-4), a branching enzyme from the alga *Cyanidium caldarium.* Boyer and Preiss (1977, Biochemistry, 16, 3693-3699), a branching enzyme from *Escherichia coli;* Baecker, et al. (1986, J. Biol. Chem. 261, 8738-8743), a gene encoding an *E. coli* branching enzyme; Kiel, J.A.K.W., et al., (1992, J. DNA Sequencing and mapping, 3, 221-232), a gene encoding a *Bacillus caldolyticus* branching enzyme; Takata, H., et al. (1994, Appl. Environm. Microbiol. 60, 3096-3104) a gene encoding a *Bacillus stearothermophilus* branching enzyme; and Kiel, et al. (1989, Gene 78, 9-17), a gene encoding a branching enzyme from a *Synechococcus* species.

GB 2 095 681 discloses a process for the production of a branching enzyme from *Bacillus* and a method of preparing an improved food product by use of such an enzyme. It is stated that the enzyme may be useful in improving the shelf life of certain food products, including bread.

EP 418 945 discloses a thermostable branching enzyme isolated and cloned from *Bacillus stearothermophilus.* The enzyme is stated to be useful for modification of starch-like materials such as starch, amylose, amylopectin, dextrin, and other polyglucose materials, by introduction of additional branches into said materials. It is stated that the enzyme may be used for the production of food or feed products containing the modified starch-like materials, but no advantages of such use are described or indicated.

It is the object of the present invention to provide a novel approach for achieving enzymatic improvements in dough quality and/or the baked product prepared therefrom.

BRIEF DISCLOSURE OF THE INVENTION

Accordingly, in a first aspect the present invention rela-

tes to a bread-improving and/or a dough-improving composition comprising an effective amount of a DGTase.

In the present context the terms "bread-improving composition" and "dough-improving composition" are intended to indicate compositions which, in addition to the enzyme component, may comprise other substances conventionally used in baking to improve the properties of dough and/or baked products. Examples of such components are given below.

The term "an effective amount" is intended to indicate an amount of enzyme which is sufficient for providing a measurable effect on the parameter of interest. For example, it is an amount resulting in a detectable change of at least one of the properties improved according to the present invention; in particular, at least one of the properties believed to contribute to staling (*vide* the first paragraph of the section Detailed Description of the Invention below).

In a second aspect, the present invention relates to a method of improving dough properties and/or properties of a baked product prepared from dough, which method comprises, in the dough making process, to add an effective amount of a DGTase to the dough or dough ingredients and subject the resulting dough to baking under suitable conditions.

As used herein, the properties of dough and/or a baked product prepared from dough said to be improved by the method of the invention includes any property which may be improved by the action of the DGTase. Important examples are an increased volume, an improved freshness (in terms of antistaling) and an improved structure and softness, as well as improved organoleptic qualities, of the baked product. Also important is increased dough stability (i.e., a less sticky dough), thereby leading to improved machinability of the dough. The improved machinability is of particular importance in connection with dough which is to be processed industrially. The improved properties may, of course, be evaluated by comparison with dough and/or baked products prepared without addition of DGTase in accordance with the present invention.

In still further aspects, the present invention relates to a dough and a baked product, respectively, produced by the

present method as well as to a pre-mix comprising an effective amount of a DGTase or a bread-improving or dough-improving composition of the invention.

In the present context, the term "pre-mix" is intended to be understood in its conventional meaning, i.e. as a mix of baking agents, normally including flour, which may be used not only in industrial bread-baking plants/facilities, but also in retail bakeries.

In a final aspect, the present invention relates to the use of a DGTase as defined herein to improve one or more properties of dough and/or a baked product prepared from dough, including (1) preventing or reducing the staling of baked products, in particular, bread; (2) increasing the loaf volume of a baked product; and (3) improving the organoleptic qualities of a baked product.

DETAILED DISCLOSURE OF THE INVENTION

According to the literature staling of bread during storage has been described to involve crumb firming, loss of crumb elasticity, reduced slice-ability, reduced palatability and reduced flavour. All of these changes are believed to be caused by the properties of the starch fraction present in bread. By the modification of the starch fraction which is achieved in accordance with the present invention, i.e. the *in situ* modification of amylose and amylopectin, it is expected that it is possible to reduce the retrogradation and thus staling of baked products substantially.

It is also believed that the modification of the starch fraction by use of the present invention results in increased volume in baked products and improved organoleptic qualities, such as flavour, mouth feel, palatability, aroma and crust colour.

Preferably, the DGTase to be used in the present invention is active at a pH in the range of 3 to 7, preferably with a pH optima in the range of 5 to 7. The enzyme may be active during the dough preparation and/or the baking process, and it is presently preferred that the enzyme is active during

both process steps. Preferably, the enzyme has an optimum activity above 45°C, more preferably above 55°C such as in the range of 45-90°C, and more preferably 55-90°C. Expressed in functional terms, it is preferred that on the one hand the enzyme is inactivated during the baking process and on the other hand the enzyme has a substantial activity above the temperature at which endo-acting amylases, such as α-amylases, present in flour are active. The latter is based on the fact that the product obtained by the action of the DGTase is degraded by endo-acting amylases.

In addition to the above mentioned properties, it may be desirable that the DGTase has a substantial oxidation stability since a number of oxidation agents are present in the dough.

It is presently contemplated that the origin of the DGTase to be used in the present invention is not critical as long as the enzyme in question has the properties mentioned above. Thus, the DGTase may be of any origin, including mammalian, plant and microbial (including bacterial or fungal) origin. A specific example of a DGTase contemplated to be of use in the present invention is the potato DGTase described by Takaha et al., J. Biol. Chem. vol. 268, 1391-1396, 1993. Said reference also discloses the recombinant production of the enzyme in an *Eschericia coli* and a *Bacillus subtilis* host cell.

The DGTase may be obtained from the organism in question by use of any suitable technique, and in particular by use of recombinant DNA techniques as known in the art. The use of recombinant DNA techniques normally comprises cultivation of a host cell transformed with a recombinant DNA vector capable of expressing and carrying a DNA sequence encoding the enzyme in question, in a culture medium under conditions permitting the expression of the enzyme and recovering the enzyme from the culture. The DNA sequence may be of genomic, cDNA or synthetic origin, or any mixture of these, and may be isolated or synthesised in accordance with methods known in the art. The enzyme may also be extracted from the organism or relevant part thereof by which it is produced in nature.

It is presently contemplated that favourable results may be obtained when the DGTase is used together with other enzymes. Thus, the bread- and/or dough-improving composition of the invention may comprise an effective amount of one or more additional enzymes. Analogously, such additional enzyme(s) may be added together with the DGTase in the methods of the invention. Examples of such additional enzymes are a cellulase; a glycosyltransferase, in particular, 1,4-α-glucan branching enzyme (E.C. 2.4.1.18); a hemicellulase, e.g., a pentosanase such as xylanase (useful for the partial hydrolysis of pentosans which increases the extensibility of the dough); a lipase (useful for the modification of lipids present in the dough or dough constituents so as to soften the dough); an oxidase, e.g. a glucose oxidase; a peroxidase (useful for improving the dough consistency); a protease (useful for gluten weakening, in particular when using hard wheat flour); a peptidase; a transglutaminase and/or an amylololytic enzyme, in particular an amylolytic enzyme without any α-1,4-endo-activity such as an α-1,4-exoglucanase or and α-1,6-endoglucanase, e.g. a β-amylase, an amyloglucosidase, a maltogenic amylase, a cyclodextrin glucanotransferase (CGTase) or the like.

The other enzyme components may be of any origin, including mammalian and plant, and preferably of microbial (including bacterial or fungal) origin. The enzymes may be obtained by conventional techniques used in the art as mentioned above.

Specific examples of branching enzymes contemplated for use in the present invention are the branching enzymes referred to above in the "Background of the Invention" section, in particular the enzyme described in EP 418 945.

Also of particular interest are the maltogenic amylase commercially available from Novo Nordisk A/S as Novamyl®, the antistaling agents Stalingase(TM) available from Gist-brocades N.V., Grindamyl MaxLife (TM) and other products of the product line Grindamyl (TM) available from Grindsted Products, products of the product line Veron (TM) available from Röhm GmbH, the glucose oxidase available from Novo

Nordisk A/S as Gluzyme®, and the lipase available from Novo Nordisk A/S as Novozym® 677.

Transglutaminase may be used as described in EP 492 406.

The enzyme(s) to be used in the present invention may be in any form suited for the use in question, e.g. in the form of a dry powder or granulate, in particular a non-dusting granulate, a liquid, in particular a stabilised liquid, or a protected enzyme. Granulates may be produced, e.g. as disclosed in US 4,106,991 and US 4,661,452 (both to Novo Industri A/S), and may optionally be coated by methods known in the art. Liquid enzyme preparations may, for instance, be stabilised by adding nutritionally acceptable stabilisers such as a sugar, a sugar alcohol or another polyol, lactic acid or another organic acid according to established methods. Protected enzymes may be prepared according to the method disclosed in EP 238 216.

Normally for inclusion in pre-mixes or flour, it is advantageous that the enzyme(s) is/are in the form of a dry product, e.g. a non-dusting granulate, whereas for inclusion together with a liquid it is advantageously in a liquid form.

In addition or in an alternative to other enzyme components, the dough-improving and/or bread-improving composition may comprise a conventionally used baking agent, e.g. one or more of the following constituents: a milk powder (to provide crust colour), gluten (to improve the gas retention power of weak flours), an emulsifier (to improve dough extensibility and to some extent the consistency of the resulting bread), granulated fat (for dough softening and consistency of bread), an oxidant (e.g. ascorbic acid, potassium bromate, potassium iodate or ammonium persulfate; to strengthen the gluten structure), an amino acid (e.g. cysteine), a sugar, and salt (e.g. sodium chloride, calcium acetate, sodium sulfate or calcium sulphate; to make the dough firmer), flour or starch. Such components may also be added directly to the dough in accordance with a method of the invention.

Examples of suitable emulsifiers are mono- or diglycerides, diacetyl tartaric acid esters of mono- or diglycerides, sugar esters of fatty acids, polyglycerol esters of

fatty acids, lactic acid esters of monoglycerides, acetic acid esters of monoglycerides, polyoxyethylene stearates, phospholipids and lecithin.

The bread-improving and/or dough improving composition of the invention is typically included in the dough in an amount corresponding to 0.01-5%, in particular 0.1-3%.

In accordance with the method of the invention, in which a DGTase, optionally in combination with other enzymes as described above, is used for the preparation of dough and/or baked products, the enzyme(s) may be added as such to the mixture from which the dough is made or to any ingredient, e.g. flour, from which the dough is to be made. Alternatively, the enzyme(s) may be added as a constituent of a dough-improving and/or a bread-improving composition as described above, either to flour or other dough ingredients or directly to the mixture from which the dough is to be made.

The dosage of the enzyme(s) to be used in the method of the present invention should be adapted to the nature and composition of the dough in question as well as to the nature of the enzyme(s) to be used. Normally, the enzyme preparation is added in an amount corresponding to 0.01-1000 mg enzyme protein per kg of flour, preferably 0.1-100 mg enzyme protein per kg of flour, more preferably 0.1-10 mg enzyme protein per kg of flour.

In terms of enzyme activity, the appropriate dosage of a given DGTase, optionally in combination with other enzyme(s), for exerting a desirable antistaling effect of a baked product will depend on the enzyme(s) and the enzyme substrate(s) in question. The skilled person may determine a suitable enzyme unity dosage on the basis of methods known in the art.

When one or more additional enzyme activities are to be added in accordance with the method of the invention, these activities may be added separately or together with the DGTase, optionally as constituent(s) of the bread-improving and/or dough-improving composition of the invention. The other enzyme activities may be any of the above described

enzymes and may be dosed in accordance with established baking practice.

As mentioned above, the DGTase, optionally in combination with other enzyme(s) as described above, is added to any mixture of dough ingredients, to the dough, or to any of the ingredients to be included in the dough; in other words, the enzyme(s) may be added in any step of the dough preparation and may be added in one, two or more steps, where appropriate.

The handling of the dough and/or baking is performed in any suitable manner for the dough and/or baked product in question, typically including the steps of kneading the dough, subjecting the dough to one or more proofing treatments, and baking the product under suitable conditions, i.e. at a suitable temperature and for a sufficient period of time. For instance, the dough may be prepared by using a normal straight dough process, a sour dough process, an overnight dough method, a low-temperature and long-time fermentation method, a frozen dough method, the Chorleywood Bread process, or the Sponge and Dough process.

The dough and/or baked product prepared by the method of the invention are normally based on wheat meal or flour, optionally in combination with other types of meal or flour such as corn flour, rye meal, rye flour, oat flour or meal, soy flour, sorghum meal or flour, or potato meal or flour.

In the present context the term "baked product" is intended to include any product prepared from dough, either of a soft or a crisp character. Examples of baked products, whether of a white, light or dark type, which may be advantageously produced by the present invention are bread (in particular white, whole-meal or rye bread), typically in the form of loaves or rolls, French baguette-type bread, pita bread, tacos, cakes, pan-cakes, biscuits, crisp bread and the like.

The dough of the invention may be of any of the types discussed above, and may be fresh, frozen or pre-baked. The preparation of frozen dough is described by K. Kulp and K. Lorenz in "Frozen and Refrigerated Doughs and Batters".

From the above disclosure, it will be apparent that the dough of the invention is normally a leavened dough or a dough to be subjected to leavening. The dough may be leavened in various ways, such as by adding sodium bicarbonate or the like, or by adding a leaven (fermenting dough), but it is preferred to leaven the dough by adding a suitable yeast culture, such as a culture of *Saccharomyces cerevisiae* (baker's yeast). Any of the commercially available *S. cereviciae* strains may be employed.

As mentioned above, the present invention further relates to a pre-mix, e.g., in the form of a flour composition, for dough and or baked products made from dough, which pre-mix comprises a DGTase and optionally other enzymes as specified above. The pre-mix may be prepared by mixing enzyme preparation(s) comprising the relevant enzyme(s) or a bread-improving and/or dough-improving composition of the invention comprising the enzyme(s) with a suitable carrier such as flour, starch, a sugar or a salt. The pre-mix may contain other dough-improving and/or bread-improving additives, e.g., any of the additives, including enzymes, mentioned above.

Techniques which can be used to determine improvements achieved by use of the present invention are described below. The organoleptic qualities mentioned above may be evaluated using procedures well-established in the baking industry, and may include, for example, the use of a panel of trained taste-testers.

MATERIALS AND METHODS

Determination of DGTase activity

The DGTase activity may be determined as described in EP 675 137.

Preparation of bread

According to the present invention the effect of adding a DGTase may be tested in doughs and breads as follows:

White bread may be prepared from the following basic recipe:

Basic recipe

Wheat flour	100 %
Salt	1.5 %
Yeast (fresh)	5.0 %
sugar	1.5 %
Water	58 %

Preparation of bread

Procedure:

1. Dough mixing (Spiral mixer)
   3 min. at 625 RPM
   3.5 min. at 1250 RPM
   the mixing time is determined and adjusted by a skilled baker so as to obtain an optimum dough consistence under the testing conditions used.
2. 1st proof: 15 min. at room temperature (about 22 °C), covered by a cloth
3. Scaling and shaping;
4. Final proof: 32°C - 82% RH, 55 min.;
5. Baking: 235°C, 22 min. for rolls and 35 min for loaf.

Evaluation of Dough and Baked Products

Dough and baked products may be evaluated as follows:

Loaf specific volume: the mean value of 4 loaves volume are measured using the traditional rape seed method. The specific volume is calculated as volume ml per g bread. The specific volume of the control (without enzyme) is defined as 100. The relative specific volume index is calculated as:

$$\text{Specific vol. index} = \frac{\text{specific vol. of 4 loaves}}{\text{spec. vol. of 4 control loaves}} * 100$$

The dough stickiness and crumb structure: may be evaluated visually according to the following scale:

Dough stickiness:	almost liquid	1
	too sticky	2
	sticky	3
	normal	4
	dry	5
Crumb structure:	very poor	1
	poor	2
	non-uniform	3
	uniform/good	4
	very good	5

Staling properties of Baked Products: is determined on bread, e.g. on day 1, 3, 7 and 9 after baking. Evaluation of staleness and texture can be done according to AACC method 74-09.

The principles for determination of softness and elasticity of bread crumb are as follows:

1. A slice of bread is compressed with a constant speed in a texture analyzer, measuring the force for compression in g.
2. The softness of the crumb is measured as the force at 25% compression.
3. The force at 40% compression (P2) and after keeping 40% compression constant for 30 sec. (P3) is measured and the ratio (P3/P2) is the elasticity of the crumb.

CLAIMS

1. A bread-improving or a dough-improving composition comprising an effective amount of a DGTase.

2. A bread-improving or dough-improving composition according to claim 1, in which the DGTase is of plant or microbial origin.

3. The bread-improving or dough-improving composition according to claim 1, which further comprises an effective amount of another enzyme, such as a cellulase, a hemicellulase, a glycosyltransferase, a pentosanase, a lipase, a peroxidase, an endo-protease, an oxidase, a peptidase, a transglutaminase and/or an amylolytic enzyme.

4. The bread-improving or dough-improving composition according to claim 3, in which the glycosyltransferase is a 1,4-alpha-glucan branching enzyme.

5. The bread-improving or dough-improving composition according to claim 3, in which the amylolytic enzyme is selected from the group consisting of α-amylase, β-amylase, maltogenic α-amylase, amyloglucosidase and CGTase.

6. A bread-improving or dough-improving composition according to claim 3, in which the enzyme is of plant or microbial origin.

7. A bread-improving or dough-improving composition according to any of claims 1-6, which further comprises another bread- or dough-improving agent.

8. A method of improving one or more properties of dough and/or a baked product prepared from dough, which method comprises, in the dough making process, to add an effective amount of a DGTase, according to any of claims 1-7, to the dough or dough ingredients and subject the resulting dough to

baking under suitable conditions.

9. The method according to claim 8, in which an effective amount of another enzyme, such as a cellulase, a hemicellulase, a glycosyltransferase, a pentosanase, a lipase, a peroxidase, an endo-protease, an oxidase, a peptidase, a transglutaminase and/or an amylolytic enzyme, is added to the dough or dough ingredients.

10. The method according to claim 8 or 9, in which the enzyme(s) is/are added in the form of a bread-improving or dough-improving composition as defined in any of claims 1-7.

11. The method according to any of claims 8 to 10, in which the DGTase is added in an amount corresponding to 0.01-1000 mg enzyme protein per kg of flour.

12. A baked product or a dough prepared by the method according to any of claims 8 to 11.

13. A pre-mix for dough comprising an effective amount of a DGTase or a bread-improving or dough-improving composition according to any of claims 1-7.

14. Use of a DGTase for improving one or more properties of dough and/or a baked product prepared from dough selected from the group consisting of:

(a) increasing loaf volume;

(b) preventing or reducing staling; and

(c) improving organoleptic qualitites of a baked product.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 97/00201

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A21D 8/04
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A21D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0675137 A2 (EZAKI GLICO CO., LTD.), 4 October 1995 (04.10.95) --	1-14
A	EP 0687414 A1 (GIST-BROCADES B.V.), 20 December 1995 (20.12.95) --	1-14

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

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Information on patent family members

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